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REVIEWS

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Ribosome: Lessons of a Molecular Factory Construction

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Abstract—The ribosome is a macromolecular complex responsible for protein biosynthesis. Two subunits of the bacterial ribosome contain three RNA molecules of more than 4000 nt in total and more than 50 proteins. Ribosome assembly is an intricate multistep process, which is vital for the cell. The review summarizes the current concepts of the mechanisms sustaining bacterial ribosome assembly in the cell and in vitro model systems. Some details of assembling this machine are still unknown.

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INTRODUCTION

The ribosome is the most widespread molecular machine that synthesizes proteins as its main function. Ribosomal RNA accounts for a major portion of total cell RNA. The ribosome consists of a small (Fig. 1a) and large (Fig. 1b) subunits and dissociates into them reversibly. In *Escherichia coli* the subunits are named 50S and 30S according to their sedimentation coefficients [1]. A prokaryotic ribosome is approximately $20 \times 17 \times 17$ nm in dimensions, and its small subunit is approximately 23 nm in length and 12 nm in width.

The 16S rRNA is a main component of the small ribosomal subunit and consists of 1542 nt (Fig. 2a). Apart from the 16S rRNA, the small subunit includes 21 proteins, which are termed S1–S21 in the order of increasing electrophoretic mobility [2]. The large subunit includes two rRNAs, 23S (2904 nt) and 5S (120 nt) (Fig. 2b), and 33 proteins (L1–L36). The majority of the ribosomal proteins are vital for the cell [3].

Various secondary structure domains of the 16S rRNA correspond to large spatial blocks of the 30S subunit. Thus the 5'-terminal domain forms a body and a shoulder; the central domain forms a platform, which contains the anti-Shine–Dalgarno sequence; the 3' major domain forms a head; and the 3' minor domain occurs at the interface with the 50S subunit and forms part of the body of the 30S subunit. In addition, the 30S subunit has a tunnel, which accommodates the 3'-terminal region of mRNA during translation, and a spur and a neck, which are formed by 16S rRNA helices 6 and 28, respectively. The main function of the 30S subunit is to read mRNA [4, 5].

The spatial structure of the large subunits has characteristic projections: fingers L1 and L7/L12 and a central protuberance, which is made up of the 5S rRNA and proteins L5, L18, and L25. In addition, the 50S subunit has a cleft with a catalytic peptidyltransferase center located at its bottom. A tunnel passes through the 50S subunit from the peptidyltransferase center to its cytoplasmic side to allow the exit of the nascent peptide. The tunnel is approximately 100 Å in length and 25 Å in diameter and can accommodate a peptide of 40 amino acid residues. Both of the subunits are involved in forming the A, P, and E tRNA-binding sites, which consecutively accommodate tRNA in the course of polypeptide synthesis [6]. Ribosome assembly is an intricate and well coordinated process. Its main steps are (1) rRNA synthesis, processing, and modification; (2) synthesis and modification of ribosomal proteins; (3) folding of rRNAs and ribosomal proteins; (4) binding of ribosomal proteins with rRNAs; and (5) binding of accessory proteins involved in ribosome assembly. Many of the steps occur simultaneously [7].

NUCLEOLYTIC rRNA PROCESSING

The three rRNAs (16S, 23S, and 5S) are synthesized as one transcript (Fig. 3). Transcript maturation, the formation of local secondary structure elements, and the binding of first ribosomal proteins start before rDNA transcription is complete. Certain nucleotides undergo enzymatic modification at the same time [4].

RNase III is the first endoribonuclease to cleave the rRNA transcript, separating a rRNA precursor and tRNAs. The flanking sequences of the 16S and 23S rRNAs form a double helix during transcription. This element is recognized by RNase III [8]. The cleavage

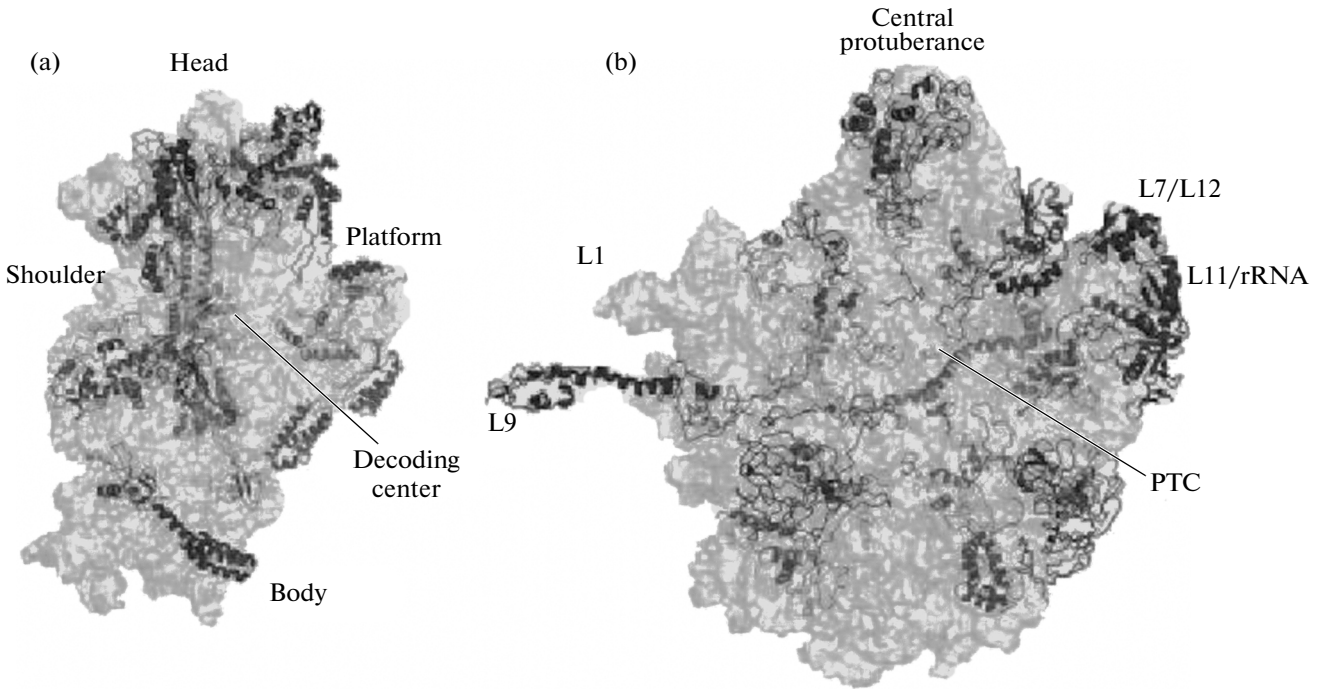


Fig. 1. Main morphological elements of the (a) small 30S (modified from [2]) and (b) large 50S subunits of the *E. coli* ribosome. The small subunit seen from the large subunit side, and the large subunit seen from the small subunit side. PTC, peptidyltransferase center.

yields precursors of the 16S (17S rRNA), 23S, and 5S (9S rRNA) rRNAs and several tRNAs. RNases E and G, together with unidentified RNases, remove 115 nt from the 5' end and 33 nt from the 3' end of the rRNA transcript, thus producing the 16S rRNA. RNase III initiates maturation of the 23S rRNA. The 23S rRNA excised by RNase III has extra nucleotides: three or seven at the 5' end and seven or nine at the 3' end [9]. A still unknown enzyme ultimately processes the 5' end, while RNase T (an exoribonuclease) is responsible for maturation of the 3' end. One or two tRNA sequences are still left 3' of the 5' rRNA precursor after RNase III cleavage. The 5' tRNA end is processed by RNase P, producing the 9S rRNA with 84 extra nucleotides at the 5' end and 42 extra nucleotides at the 3' end [10]. The processing is completed by RNase T and a still unidentified RNase (Fig. 3) [11–13].

ASSEMBLY OF THE 30S RIBOSOMAL SUBUNIT

Proteins interacting with the 16S rRNA are classed by target rRNA region or order of binding. The proteins that directly interact with rRNA are termed primary, then secondary proteins bind, and tertiary proteins are the last to bind [7].

To describe the formation of the 30S subunit, a scheme was proposed on the basis of Nomura's reconstruction of the 30S subunit in vitro (Fig. 4a). A superimposition of the scheme with the spatial structure of the ribosome is shown in Fig. 5a.

The proteins that directly bind to the 16S rRNA include S4, S7, S8, S15, S17, and S20. A complex of S4 with the 5' region of the 16S rRNA can form at a lower temperature. Experiments with chemical rRNA modification (footprinting) showed that heating makes the complex to undergo conformational changes that stabilize the S4–RNA interaction and facilitate the binding of secondary S16 and tertiary S12 (Fig. 4). When S4 and the 16S rRNA are heated separately and then combined, the conformation does not change, and the subsequent proteins do not bind [14].

A similar interaction hierarchy was observed for the binding of other ribosomal proteins. It is possible to conclude that one of the functions performed by the primary proteins is to ensure the RNA conformation favorable for the binding of the next proteins. Ribosomal proteins mutually affect each other's binding sites even when binding independently, as was shown with the example of S8 and S15 [15]. A study of the S20 surrounding showed that S20 initially forms contacts with the 5' rRNA domain and interacts with the 3' minor domain (helix H44–nucleotide C1399) at the end of assembly according to the assembly direction from the 5' to the 3' end of the 16S rRNA [16].

Intermediates of in vivo assembly of the 30S subunit are difficult to detect in normally growing cells. Initial studies of the intermediates were employed ultracentrifugation of the cytoplasm from strains car-

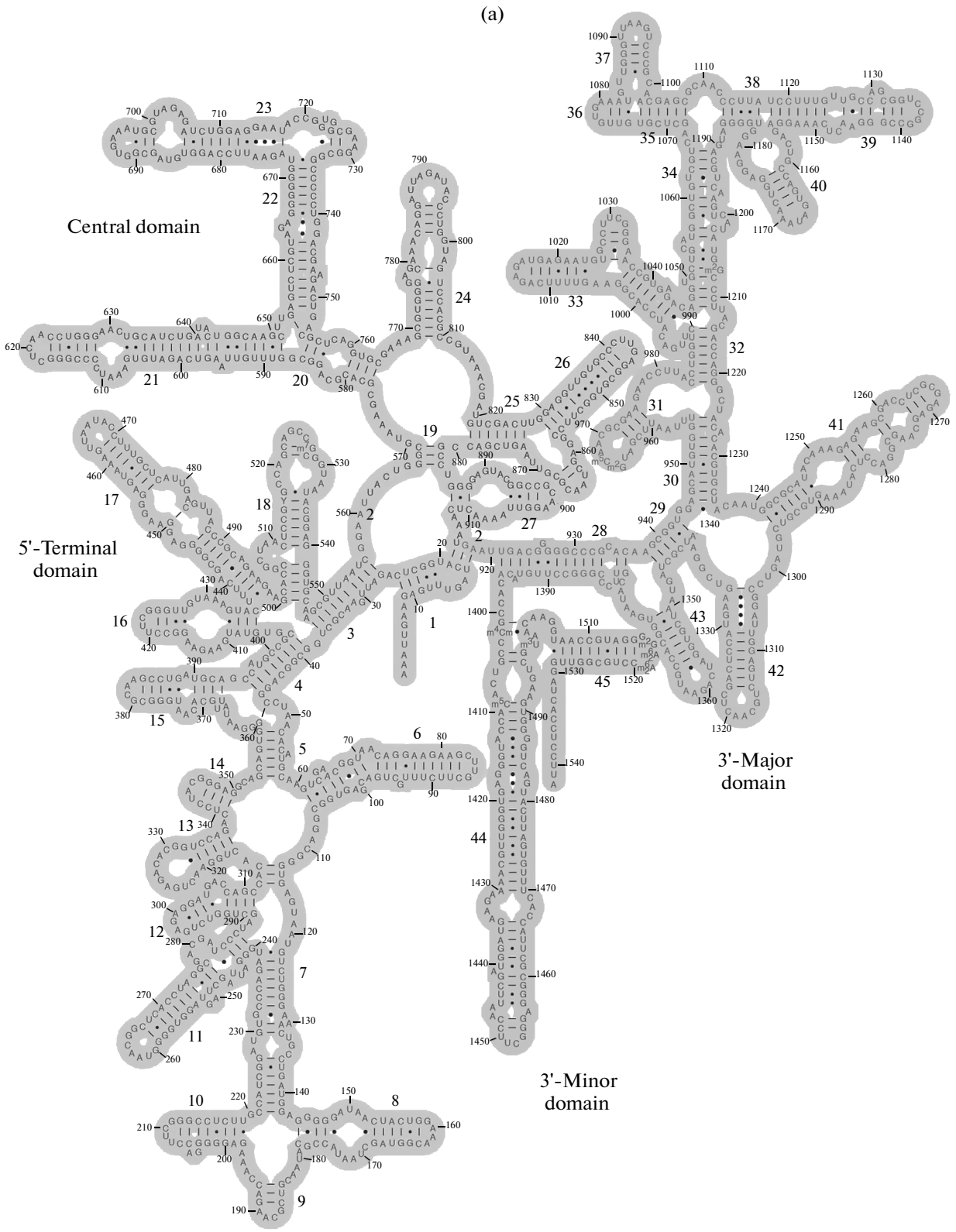
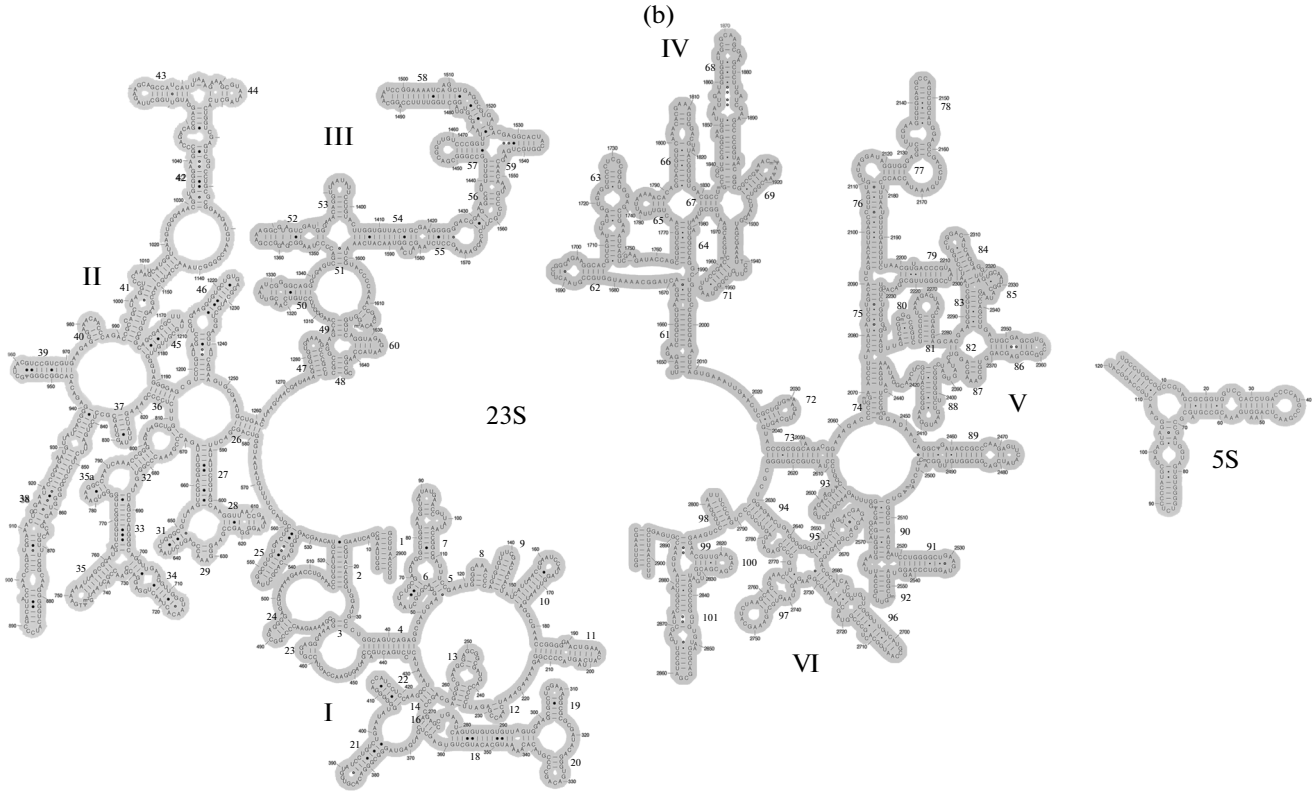


Fig. 2. Secondary structures of the *E. coli* (a) 16S, (b) 23S, and 5S rRNAs. Secondary structure domains are indicated (modified from [4, 5]).



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rying cold- or temperature-sensitive mutations of the ribosomal component genes. In wild-type strains, the intermediates can be detected by pulse labeling with radioactive or stable isotopes. Two intermediates of 30S subunit assembly ($p_1$30S and $p_2$30S) were identified in vivo by this method (Fig. 6b) [7, 17].

Fourteen intermediates of in vivo 30S subunit assembly were revealed by cryo-electron microscopy and classed into early, intermediate, and late. The early assembly intermediates include the 16S rRNA, S4, S17, and S20, as well as S16, which is a secondary binding protein according to Nomura's in vitro findings. Thermodynamic, kinetic, and electron microscopic data made it possible to reconstruct the process of 30S subunit assembly in vivo. Assembly starts with the formation of early intermediates, which contain rapidly binding primary and secondary ribosomal proteins. Then, a second group of intermediates forms to include the primary and secondary proteins that bind slowly in the region of the central and 3' domains. A culminating point of assembly is the formation of a fourth group of intermediates via incorporation of slowly binding tertiary proteins (Fig. 4b). Intermediates of a third group, which were detected by cryo-electron microscopy, can be classed as a subtype of the fourth group, appearing during assembly of the body and head of the 30S subunit. The differences between in vivo and Nomura's in vitro data can be explained by the fact that ribosome assembly cofactors are present in the cell to minimize the formation of erroneous intermediates [18].

Further data on the intermediates of in vivo ribosome assembly were obtained using a combination of radioactive pulse labeling of ribosomes with chromato-mass spectrometry [19]. The ribosomal proteins involved in 30S subunit assembly are divided into four groups depending on the order of rRNA binding, and the division fully agrees with the electron microscopy findings [18]. S4, S6, S8, S15, S16, S17, S18, and S20 are the first to bind to the 5' and central domains of the 16S rRNA. S7 and S11 bind to the central and 3' domains, while S5 and S12, which are secondary binding proteins, bind to the 5' domain. The third group includes S9, S10, S13, S14, and S19. The fourth group consists of S2, S3, and S21. S20 belongs to primarily binding proteins in vitro, but is one of the last to interact with the 30S subunit in vivo (Fig. 4c) [19].

Two intermediates, which form at different temperatures, were observed when reconstructing the 30S ribosomal subunit in vitro. Intermediate RI, which has a sedimentation coefficient of 21S–22S and consists of the 16S rRNA and 15 ribosomal proteins, is detectable at lower temperatures (0–15°C). Reconstruction of the 30S subunit cannot be completed until the tem-

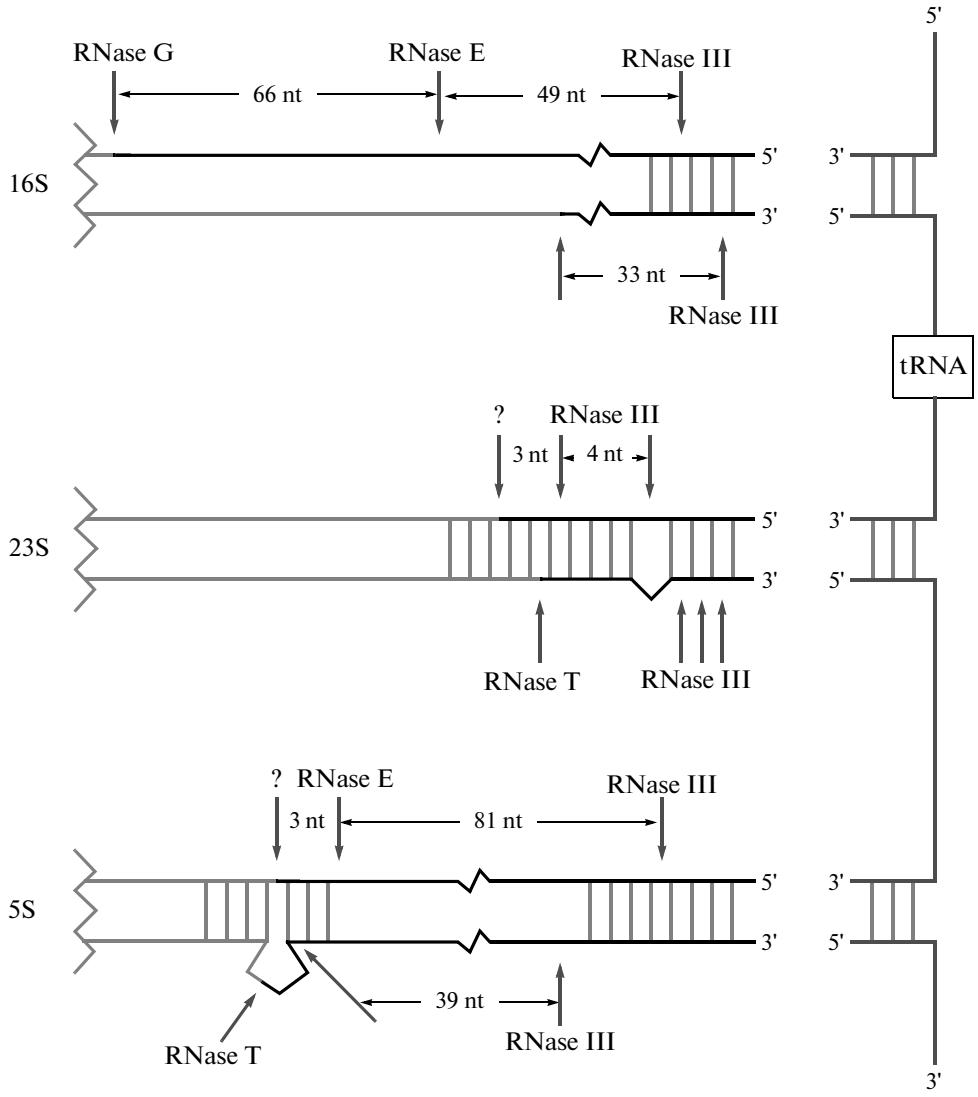


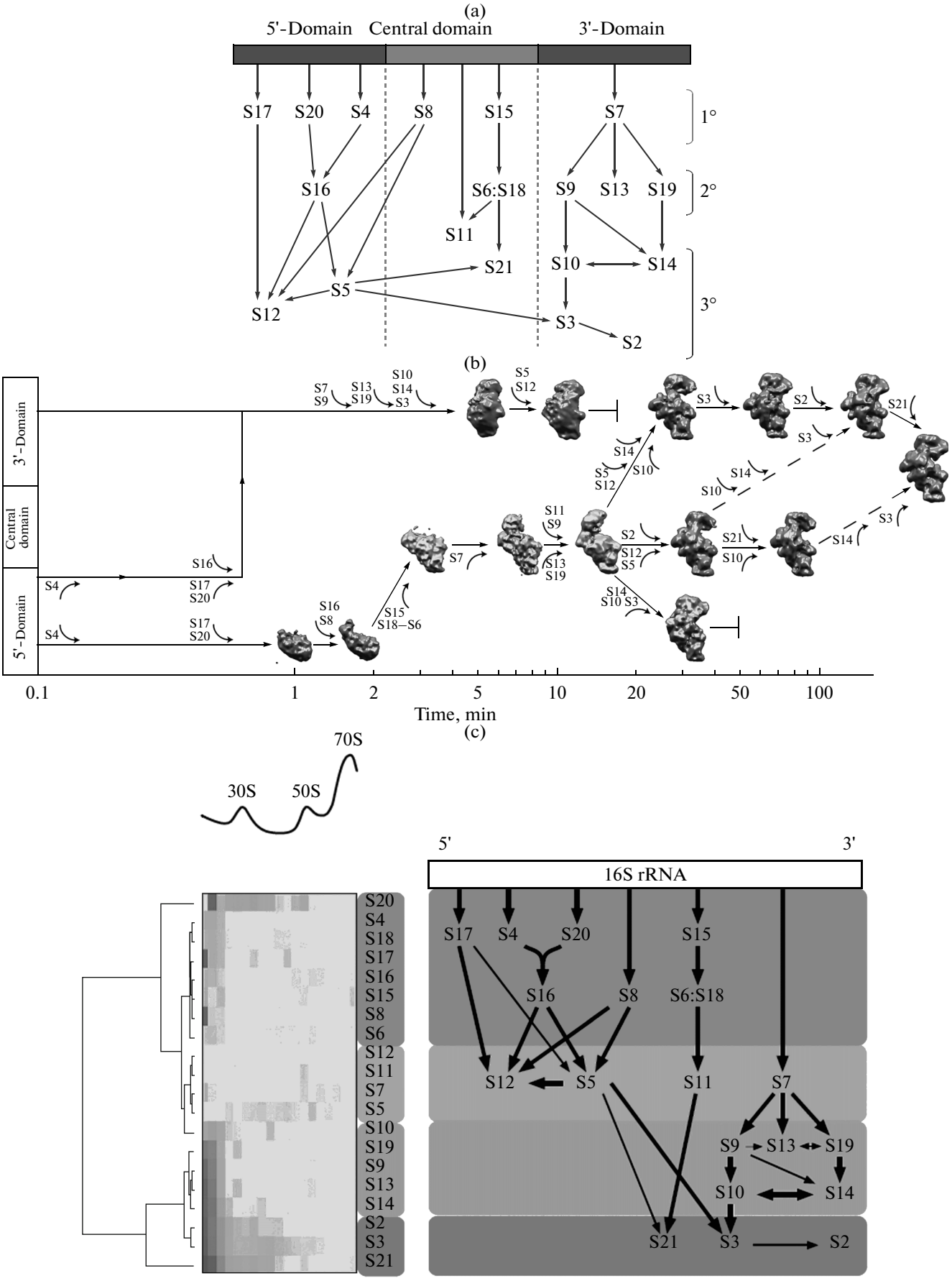
Fig. 3. Maturation of rRNAs. The rRNA transcript synthesized as a single precursor is cleaved by several RNases. The cleavage sites of known nucleases are indicated with arrows; the enzymes are shown. The cleavage sites of still unidentified enzymes are indicated with (?) (modified from [13]).

perature is increased to 40°C. At 40°C, additional proteins interact with intermediate RI, conformational changes occur, and intermediate RI* with a sedimentation coefficient of 25S–26S forms. The remaining proteins bind to intermediate RI* to produce the active 30S subunit (Fig. 6a) [20].

Assembly of ribosomal subunits in vitro proceeds at a lower rate than in vivo. In vitro assembly can be accelerated by adding factors involved in ribosome assembly in the cell, such as Era, RimM, and RimP [21]. The assembly intermediates obtained in vivo and in vitro can differ in composition in spite of their similarity in sedimentation coefficient (Table 1) [13].

The rate of ribosomal protein binding with the 16S rRNA is discussed in [22]. Primary proteins usually bind quicker than the other proteins (rate constant 0.2–2 s^{-1}) (Fig. 5b). The proteins that interact with the 5' rRNA domain bind quicker than the proteins

Fig. 4. (a) Nomura's map of in vitro assembly of the small subunit (modified from [14]). The 16S rRNA divided into domains is shown at the top. The hierarchy of ribosomal protein interactions with the rRNA is shown below. 1, primary; 2, secondary; and 3, tertiary binding proteins are indicated. (b) In vivo 30S subunit assembly map based on thermodynamic, kinetic, and electron microscopic data (modified from [17]). (c) Map of 30S subunit assembly based on chromato-mass spectrometry data (modified from [19]).


(a)
5'-Domain
Central domain
3'-Domain
S17
S20
S4
S8
S15
S7
1°
S16
S6:S18
S9
S13
S19
2°
S11
S21
S10
S14
S5
S12
S3
S2
3°
(b)
3'-Domain
Central domain
5'-Domain
S7
S9
S13
S19
S10
S14
S3
S5
S12
S16
S4
S17
S20
S8
S15
S18–S6
S11
S2
S21
0.1
1
2
5
10
20
50
100
Time, min
(c)
70S
30S
50S
5'
3'
16S rRNA
S20
S4
S18
S17
S16
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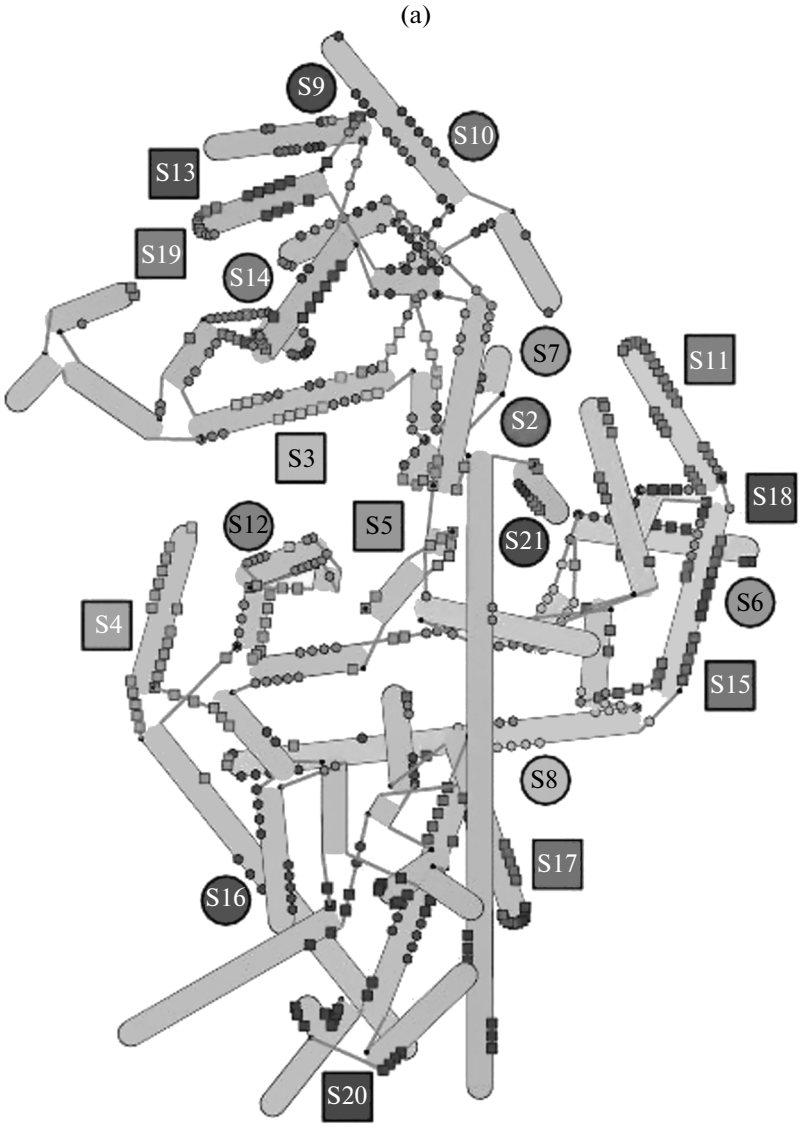
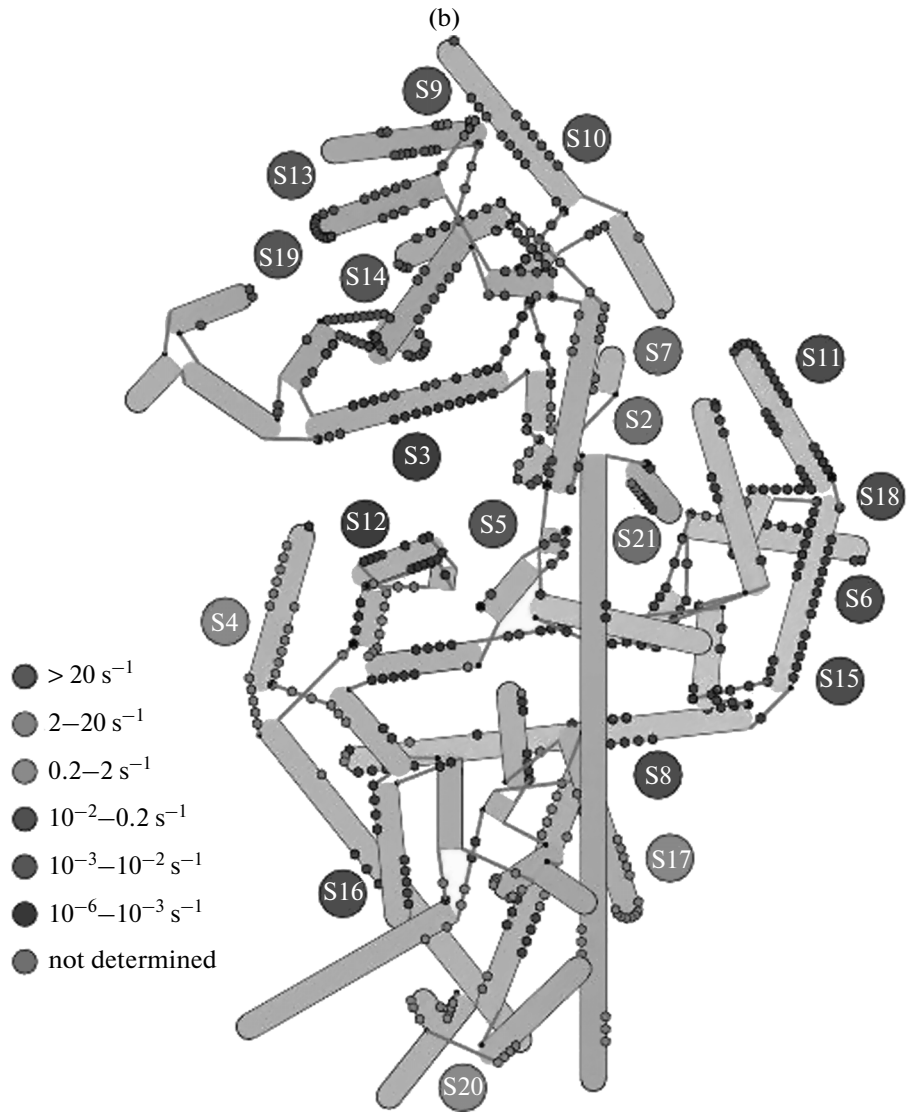


Fig. 5. Interactions of ribosomal proteins with rRNAs. (a) Specificity of contacts between ribosomal proteins and the 16S rRNA. A flat scheme of the 16S rRNA folding is shown. Ribosomal proteins are indicated. Proteins and contacting nucleotides are shown with various shades of gray. (b) Formation rates of 16S rRNA contacts with ribosomal proteins. Ribosomal proteins and interacting nucleotides are shaded according to the binding rate (modified from [17]).

that interact with the central and 3' domains, supporting the model of assembly in the 5'-to-3' direction. S5 and S12 bind to the 5' domain slower than the other proteins and do not follow the 5'-to-3' direction. This circumstance possibly reflects the fact that the proteins bind at the intercross of all rRNA domains in the vicinity of the decoding center of the 30S subunit. Another interesting example is provided by S7, which determines the subsequent binding of S9, S13, and S19. Initial S7–rRNA contacts accelerate only S9 binding, while the combination of S9 and S19 clearly increases the rate of interaction with rRNA for S9, S13, and S10.

ASSEMBLY OF THE 50S RIBOSOMAL SUBUNIT

Assembly of the 50S subunit is far more intricate than that of the 30S subunit. This is because the 23S rRNA is actually twice as long as the 16S rRNA and binds with a 1.5-fold greater set of ribosomal proteins. To further complicate the process, the 50S subunit contains not only the 23S rRNA, but also the 5S rRNA. Six domains (I–VI) are recognized in the 23S rRNA structure. This complex domain structure suggests that assembly hardly proceeds in on particular direction in this case, in contrast to 30S subunit assem-



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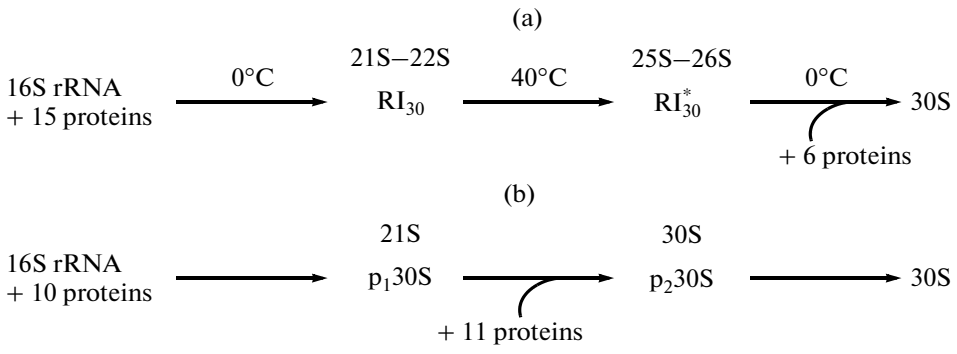

Fig. 6. Schemes of (a) in vitro and (b) in vivo assembly of the 30S subunit (modified from [17, 20]).

Table 1. Protein composition of in vivo and in vitro assembly intermediates of the 30S subunit

Protein	In vitro intermediate	In vivo intermediate
S1	–	+
S2	–	–
S3	–	–
S4	+	+
S5	+	+
S6	+	–
S7	+	–
S8	+	+
S9	+	–
S10	–	–
S11	+	–
S12	+	–
S13	+	+
S14	–	–
S15	+	+
S16	+	+
S17	+	+
S18	+	–
S19	+	–
S20	+	+
S21	–	+

bly in the 5'-to-3' direction. Nierhaus [23] mapped reconstruction of the 50S subunit (Fig. 7a) [23].

Three main domains binding with groups of proteins are recognized in the 50S-subunit rRNA by analogy with the 3', 5', and central domains of the 16S rRNA. The 23S rRNA domains are designated according to their sedimentation coefficients: 13S, 8S, and 12S. The 5S rRNA is bound during late assembly because L5, L25, and L18 are necessary for its binding.

In vitro, reconstruction of the 50S subunit proceeds through four steps. First, the 23S rRNA, 5S rRNA, and ribosomal proteins are incubated in the presence of 4 mM Mg^{2+} at 0°C to produce intermediate RI50(1), which has a sedimentation coefficient of 33S. As the mixture is then heated to 44°C, conformational changes occur, and a new 41S subparticle, which is known as RI*50(1) forms. Adding the remaining proteins results in the formation of RI50(2) with a sedimentation coefficient of 48S. At the last step, RI50(2) is incubated in the presence of 20 mM Mg^{2+} at 50°C to yield the 50S subunit (Fig. 8a). The sedimentation coefficients of the intermediates observed during in vivo assembly of the 50S subunit (Fig. 8b) are similar to those of the in vitro assembly intermediates.

Five ribosomal proteins—L4, L13, L22, and L24, which interact with the 5' region of the 23S rRNA, and L3, which binds to its 3' end—are necessary for the formation of intermediate RI*50(1). The interactions of L3 with rRNA domain IV and L24 with domain I affect the total process of 50S subunit assembly. The two proteins were shown to form two independent centers during assembly. A correct formation of the central protuberance depends on L5, L18, and L25. L20 and L24 are thought to initiate assembly of the 50S subunit [13, 24].

Pulse labeling revealed three intermediates of in vivo 50S subunit assembly; their respective sedimentation coefficients are 32S, 43S, and 50S. These precursors account for only 2–5% of the total cell RNA during logarithmic cell growth. The intermediate differ in protein composition, $p_2$50S having eight proteins more than $p_1$50S. Intermediate $p_3$50S has the same sedimentation coefficient as the 50S subunit, but the spatial rRNA fold in the intermediate still differs from that in the mature subunit. The assembly intermediates observed in vitro and in vivo differ in composition in spite of being similar in sedimentation coefficient (Table 2) [25–27].

A combination of radioactive pulse labeling of ribosomes with chromato-mass spectrometry showed that in vivo assembly of the 50S ribosomal subunit is more intricate than earlier believed [19]. Six types of assembly intermediates can be isolated. A first group contains L20, L21, L22, and L24, which bind to the 5' domain of the 23S rRNA. Intermediates of a second group contain L1, L3, L4, L13, L15, L17, and L23. Third-group intermediates form with L5, L18, L29, and L34. Intermediates of a fourth group have L14, L2, L19, and L32. Those of a fifth group have L6, L9, L11, L28, and L33. At the last assembly step, L30, L31, L35, and L36 bind to the subunit (Fig. 7b). While the 30S subunit is assembled in a directional manner (from the 5' to the 3' rRNA end), 50S subunit assembly is far less regular. Additional difficulties stem from the fact that 50S subunit assembly takes more time than assembly of the 30S subunit [19].

PROTEINS INVOLVED IN RIBOSOME ASSEMBLY

Bacterial ribosomal subparticles are possible to reconstruct from rRNAs and ribosomal proteins in a cell-free system, but the process requires nonphysiological ion concentrations and higher temperatures. The ribosome assembly rate in a cell-free system is also incomparable with that in the living cell. The cause is that many specialized proteins facilitate ribosome assembly in the cell. The proteins, which are functionally similar to chaperones, help additional structural rRNA elements to form, thus playing a necessary role

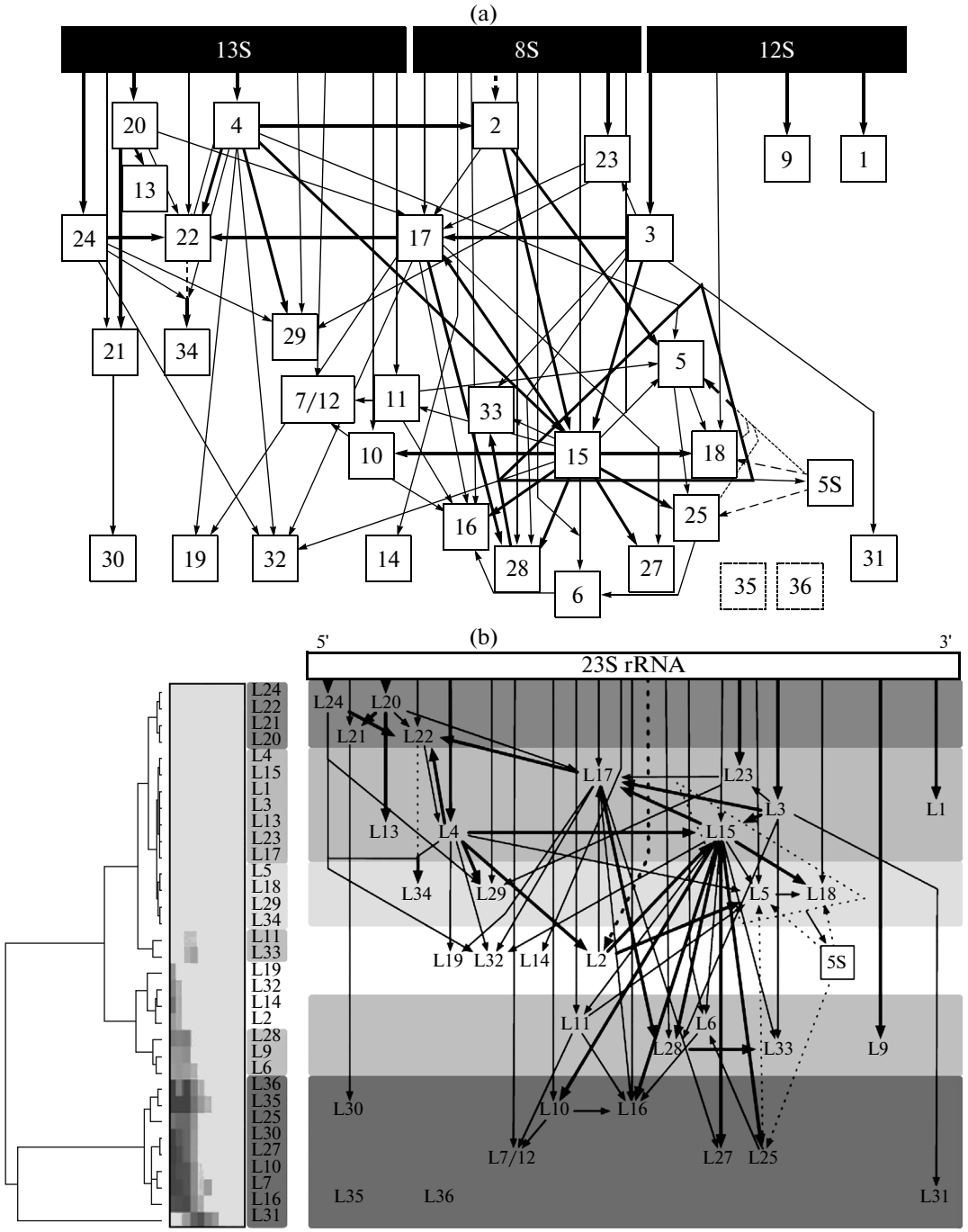

Fig. 7. (a) Scheme of 50S subunit assembly. The 23S rRNA divided into domains is shown at the top. The hierarchy of ribosomal protein interactions with the rRNA is shown below. Ribosomal proteins are shown with rectangles with corresponding numbers. The order of binding of large-subunit components is shown with arrows. 5S, 5S rRNA. Proteins interacting with the 5S rRNA are connected with it with dashed arrows. (b) Map of 50S subunit assembly based on chromato-mass spectrometry data (modified from [19]).

in ribosome assembly. The proteins provide checkpoints for ribosome assembly and thereby divide it into several steps (Fig. 9).

A combination of radioactive pulse labeling with chromato-mass spectrometry revealed 15 factors with known functions and six factors whose functions are still unknown (FkpA, YfdQ, YdiJ, YggL, YqiC, and YbhC) in ribosome assembly intermediates [19].

Assembly factors are especially important in the case of the 50S subunit because its assembly is more complex than that of the 30S subunit (Table 3; Figs. 4, 7, 9) [13].

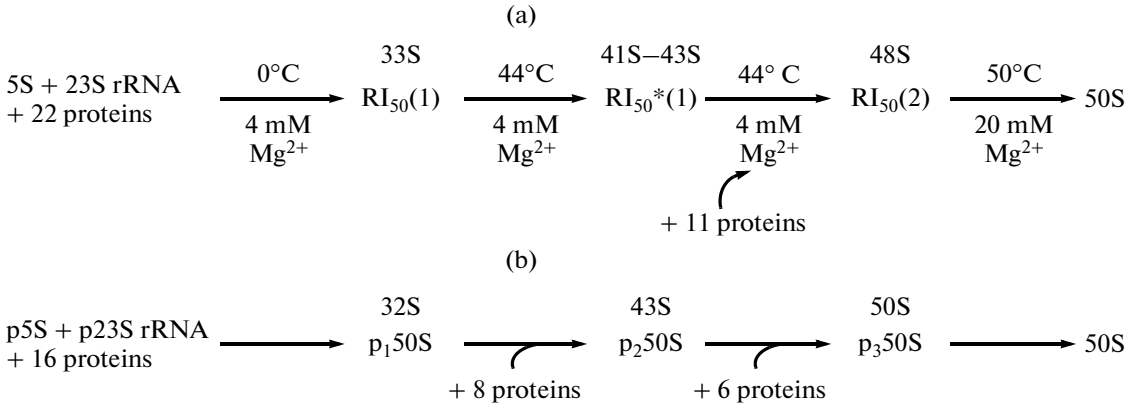

Fig. 8. Schemes of (a) in vitro and (b) in vivo assembly of the 50S subunit (modified from [13]).

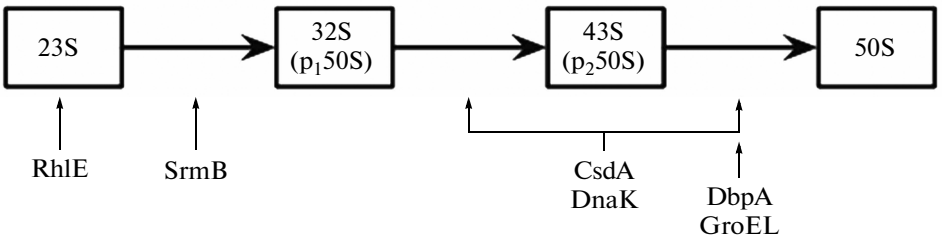

Fig. 9. Protein factors involved in 50S subunit assembly in vivo (modified from [19]).

Helicases

Assembly factors include a group of related RNA helicases with a conserved domain, which is found in both bacterial and eukaryotic proteins. The domain consists of approximately 350 amino acid residues and has nine conserved motifs, owing its name to one of them (Asp-Glu-Ala-Asp, or DEAD). In vitro studies showed that helicases possess RNA-dependent ATPase activity [28]. The helicase group possibly performs several functions, such as a local unwinding of the RNA secondary structure, a role in RNA–protein interactions, and a chaperone function. Five DEAD-box helicases—SrmB, CsdA, DbpA, RhlE, and RhlB—were identified in *E. coli*, and four of them are directly involved in ribosome biogenesis [29].

A deletion of *srmB*, whose product acts as an inhibitor of ribosomal protein L24 gene inactivation, results in a slow cell growth at lower temperatures (<30°C) and an increase in free 30S and 50S subparticles and their precursors. SrmB usually interacts with the 40S subunit, acting in early ribosome biogenesis (Fig. 9).

It is known that SrmB complexes with L4 and L24, which are necessary for the formation of the first in vitro intermediate. The CsdA heat shock protein is possibly involved in maturation of both of the ribosome subunits. CsdA suppresses the temperature-sensitive mutations of the ribosomal protein S2 gene and is necessary for the normal cell growth at temperatures below 30°C. At the same time, the protein is associated with a 50S subunit precursor and acts downstream of SrmB in biogenesis of the 50S subunit [30–32]. Lack of CsdA decelerates the cell growth, increases the amount of free subunits, and decreases the polysome amount [28–32].

A model of SrmB, CsdA, and RhlE interactions was advanced on the basis of many studies. The presence or deletion of RhlE shifts the balance either towards SrmB binding to the 50S subunit or towards CsdA binding to the 50S subunit (Fig. 10) [33].

The DbpA protein acts as an ATP-dependent helicase and is activated upon interaction with helix 92 of the 23S rRNA. A deletion of its gene exerts no effect on the bacterial cell growth rate, while its overexpression affects both the cell growth rate and ribosome assembly, increasing the amount of free subunits and changing the composition 50S assembly intermediates. DbpA is involved in the last step of ribosome assembly [29].

Chaperones

The DnaJ, DnaK, and GrpE proteins are chaperones necessary for ribosome assembly. *Escherichia coli* strains with a deletion of *dnaJ* or *dnaK* grow slower and are defective in ribosome assembly [34–36]. At temperatures higher than 42°C, mutant cells have a 30S subunit precursor with a sedimentation coefficient of 21S and two 50S subunit precursors of 32S and 45S,

and precursor maturation to functional ribosomal subunits proceeds at a lower rate. The defects are partly compensated for by overexpression of the *groEL* and *groES* heat shock genes. GroEL is necessary for complete maturation of the 45S precursor to the 50S ribosomal subunit, but is not involved in the formation of the 30S subunit [35].

The RbfA cold shock protein is necessary for cell growth at lower temperatures (Figs. 10, 11). RbfA interacts with the 5'-terminal helix of the 16S rRNA and is associated with 30S subunits, 70S ribosomes, and polysomes. Cells devoid of RbfA fail to adapt to lower temperatures, display an altered ribosome profile, and have a substantially elevated content of the 16S rRNA precursor. The phenotypic manifestation of the *rbfA* deletion is compensated for by overexpression of Era GTPase [37].

The RimM small-subunit maturation factor also facilitates the efficient processing of the 16S rRNA. A deletion of its gene leads to accumulation of 16S rRNA precursors. Other proteins involved in maturation of the 3' end of the 16S rRNA are still unknown. To play its role in maturation of the 3' rRNA end, RimM interacts with helices 31 and 33b of the 16S rRNA and ribosomal proteins S13 and S19. It is possible that RimM expedites the interactions of S13 with helix 31 and S19 with helix 33b. In a cell-free system, preincubation of RimM with the 16S rRNA improves the binding of S9, S19, S10, and S3 to the 3' domain of the 16S rRNA and, surprisingly, hinders the binding of S13 [21].

The RimP protein is encoded by the same operon as Rbfa and is involved in maturation of the 30S subunit. Mutants with a *rimP* deletion have a higher content of free subunits as compared with wild-type cells. Preincubation with RimP facilitates the binding of the 16S rRNA with S5 and S12, as well as with S9, S3, S7, and S10, which interact with the 3' domain.

RimJ acts as a suppressor of the cold-sensitive phenotype and is usually associated with ribosomal protein S5. A 30S subunit precursor provides a substrate for RimJ. It is thought that RimJ binding occurs after S4 interacts with the 5' domain and before S3 interacts with the 3' domain of the 16S rRNA [21].

GTPases

Era is a conserved GTPase that specifically binds with both 16S rRNA and the 30S ribosomal subunit in vitro (Figs. 10, 11). A deletion of its gene reduces the polysome amount and causes an accumulation of the 16S rRNA precursor in the cell. An increase in KsgA RNA methyltransferase can suppress the *era* deletion phenotype. Era ensures a twofold increase in the 16S rRNA binding rate for S9, S11, S5, and S12 and exerts a lower effect on the interactions of S13, S14, and S19 with the 3' domain of the 16S rRNA in a cell-free system [38].

Table 2. Protein composition of in vivo and in vitro assembly intermediates of the 50S subunit

Protein	In vitro intermediate (33/41S)	In vivo intermediate $p_1$50S (32S)	In vivo intermediate $p_2$50S (43S)
L1	+	+	+
L2	+	–	–
L3	+	–	+
L4	+	+	+
L5	+	+	+
L6	–	–	–
L7	+	–	+
L9	+	+	+
L10	+	+	+
L11	+	–	+
L13	+	+	+
L14	–	–	+
L15	+	–	+
L16	–	–	–
L17	+	+	+
L18	+	+	+
L19	–	–	+
L20	+	+	+
L21	+	+	+
L22	+	+	+
L23	+	–	+
L24	+	+	+
L25	–	+	+
L27	–	+	+
L28	–	–	–
L29	+	+	+
L30	–	+	+
L31	–	–	–
L32	–	–	–
L33	+	–	+
L34	+	Unknown	Unknown
L35	Unknown	Unknown	Unknown
L36	Unknown	Unknown	Unknown

RsgA is a GTPase whose activity is stimulated by 30S subunits or 70S ribosomes. In the presence of the nonhydrolyzable GTP analog guanilylimido diphosphate (GMP-PNP), ribosomal subunits occur mostly in a dissociated form, and RsgA is coprecipitated with 30S subunits. In the absence of GTP or GDP, RsgA does not bind with 30S subunits or 70S ribosomes. It is possible that the RsgA–GTP complex binds to misassembled 30S subunits contained in 70S ribosomes and induces conformational changes, leading to ribosome

Table 3. Ribosome assembly factors and the effects of deletions of their genes

Protein	Subunit or rRNA associated with assembly factor	Gene deletion phenotype		
		precursor	immature 16S rRNA	immature 23S rRNA
$CgtA_E$	30S, 50S, 16S, 23S rRNA	40S at high ionic strength	Increase	Increase
CsdA	40S	40S		Increase
DbpA	23S rRNA fragment			
Der	50S	21S, 32S, 45S	Increase	Increase
DnaK-DnaJ			Increase	
Era	16S rRNA, 30S		Increase	
GroEL-GroES				
KsgA				
RbfA	30S		Increase	
RhlE	70S			
RimJ	Pre-30S			
RimM	30S		Increase	
RimP	30S		Increase	
RrmJ		40S		Increase
RsgA	30S		Increase	
SrmB	40S	40S	Increase	Increase
YihI	40S			

dissociation into the subunits. A *rsgA* deletion decelerates the cell growth, increases the amount of free subunits, and causes an accumulation of the 16S rRNA precursor. Interestingly, a *rsgA* deletion and inhibition of GTP-hydrolyzing activity of RsgA similarly confer resistance to high salt stress. Overexpression of *era* compensates for the consequences of a *rsgA* deletion [39].

Der acts as a GTPase and belongs to the same family as Era. Der contains two domains responsible for GTP binding, which are in its N-terminal region. Both of the GTP-binding domains are inactivated at 42°C. Der interacts with 50S subunits in the presence of a nonhydrolyzable analog of GTP, but not GDP. A deletion of *der* increases the amount of dissociated subunits and leads to an accumulation of the 16S and 23S rRNA precursors [41]. Recent studies revealed the Der-associated protein YihI, which functions to stimulate GTPase activity of Der and binds with Der at a 1 : 1 ratio. YihI can be assumed to act as a Der regulator because YihI overexpression inhibits the Der function (Figs. 10, 12) and 50S subunit assembly. This is accompanied by an accumulation of nonprocessed 16S and 23S rRNAs and 5S rRNA precursors, and L9, L18, and L25 bind to the 23S rRNA less efficiently. An excess of Der compensates for the effects of YihI overexpression [40]. YihI is thought to interact with Der in the lag growth phase, causing dissociation of the Der complex with the 50S subunit. In the exponential growth phase, the YihI pool decreases, the amount of free GTP-bound Der increases, and Der promotes the completion of 50S subunit assembly [42].

Conserved GTPase CgtAE binds to both 30S- and 50S-subunit rRNAs in the presence of guanilylimido diphosphate. In vitro, CgtAE coprecipitates with the 16S and 23S rRNAs in the presence of GTP and interacts with S3, S4, S5, S13, S16, L2, L4, L16, and L17, like CsdA [43]. A deletion of *cgtAE* increases the pool of free 30S and 50S subunits, causes errors in 16S and 23S rRNA processing, and leads to a lack of L16, L33, and L34 in 50S subunits. In a cell-free system, CgtAE interacts only with mature 50S subunits and fails to bind with precursors, suggesting a role in late 50S subunit assembly in the cell [44].

MODIFICATION OF rRNA NUCLEOTIDES AND RIBOSOMAL PROTEINS

Both tRNAs and rRNAs (other than the 5S rRNA) are covalently modified during their maturation in *E. coli* cells. Modified nucleotides occur mostly in the functionally important elements of the ribosome, such as the peptidyltransferase center, tRNA-binding sites, and sites of contacts between the two subunits (Fig. 13).

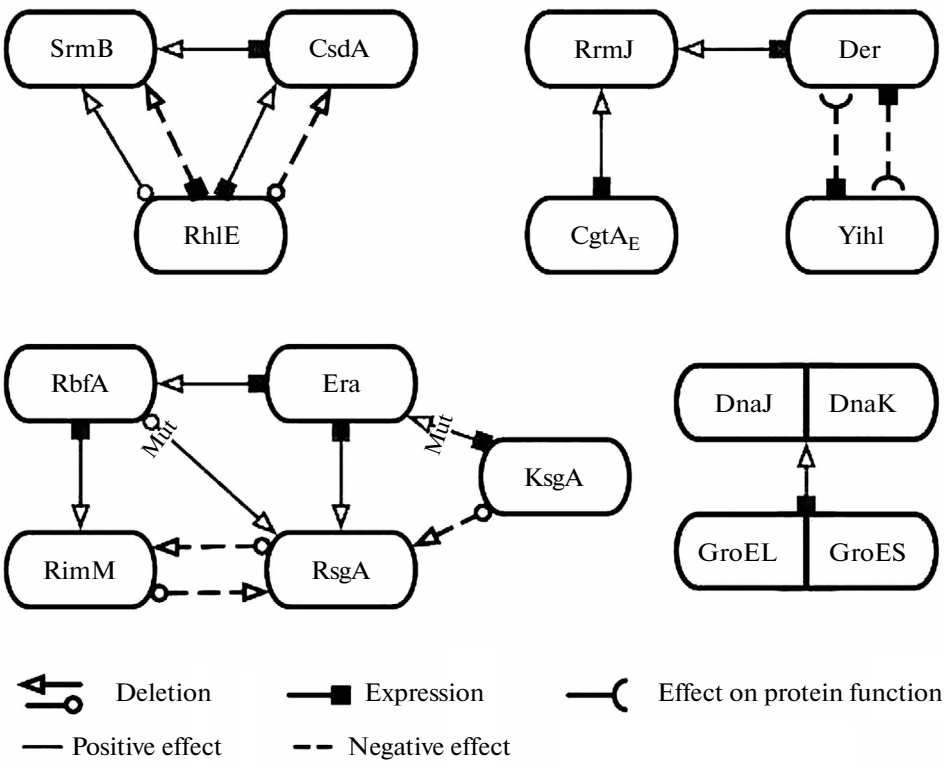

Fig. 10. Interactions of ribosome assembly factors. The types of functional interactions are shown at the bottom (modified from [33]).

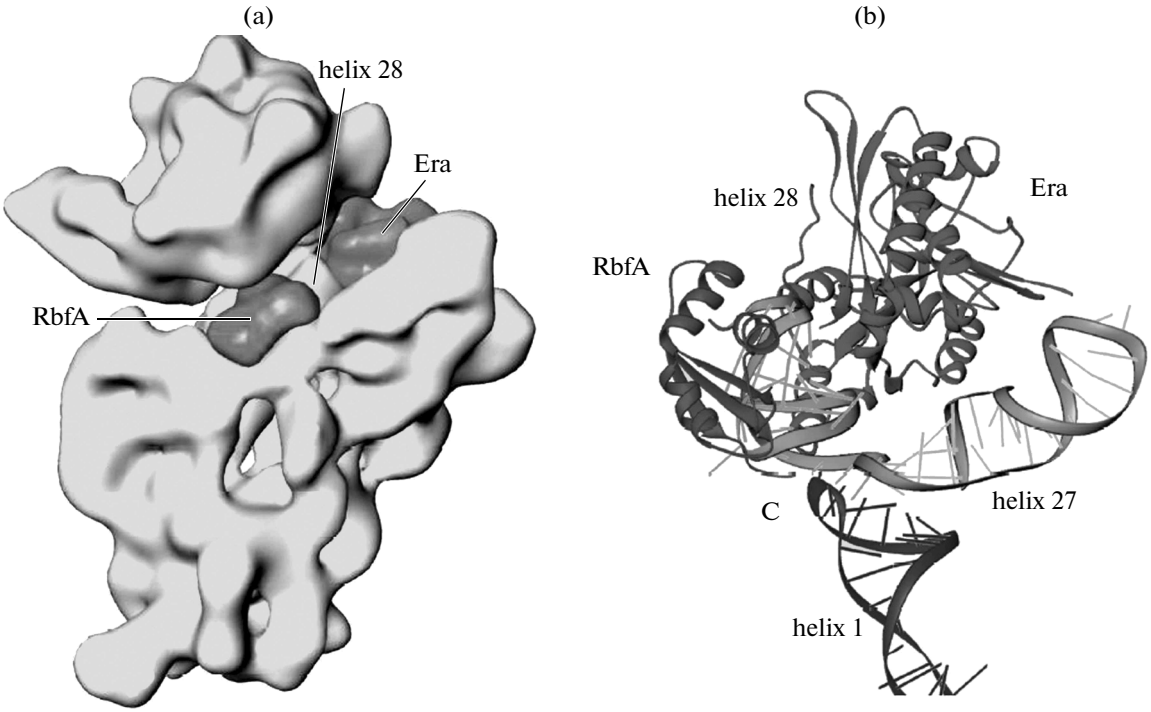

Fig. 11. Binding of RbfA and Era to the 30S ribosomal subunit. (a) The structure of the 30S-subunit complex with RbfA and Era is shown as revealed by cryo-electron microscopy. (b) Wireframe model of the RbfA and Era interactions with a 16S rRNA region. Helices 1, 27, and 28 are shown in the 30S subunit (modified from [37]).

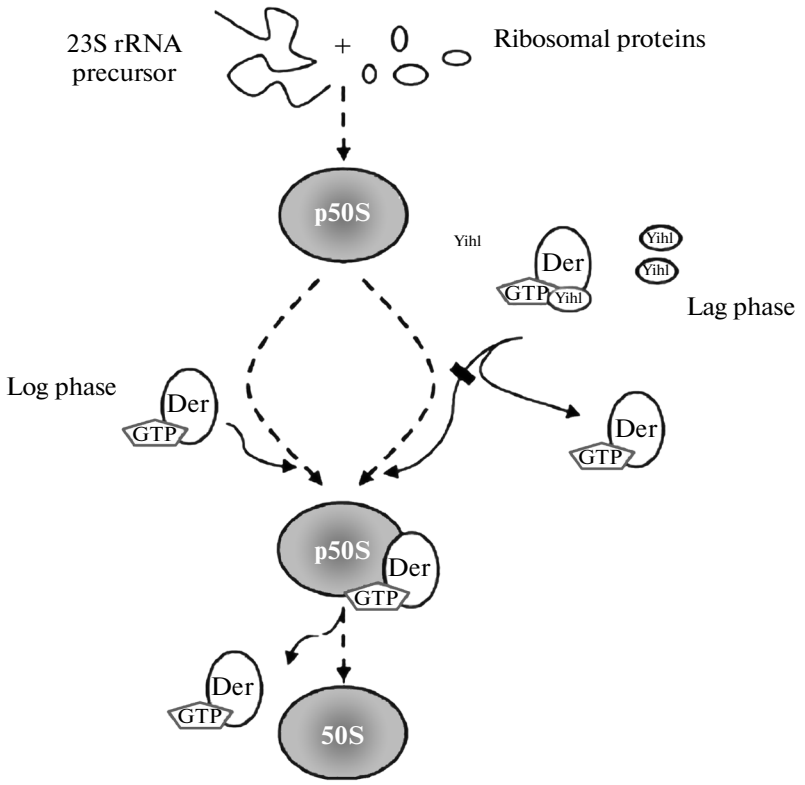

Fig. 12. Model of the roles Der and YihI play in 50S subunit biogenesis. p50S, precursor of the 50S subunit (modified from [40]).

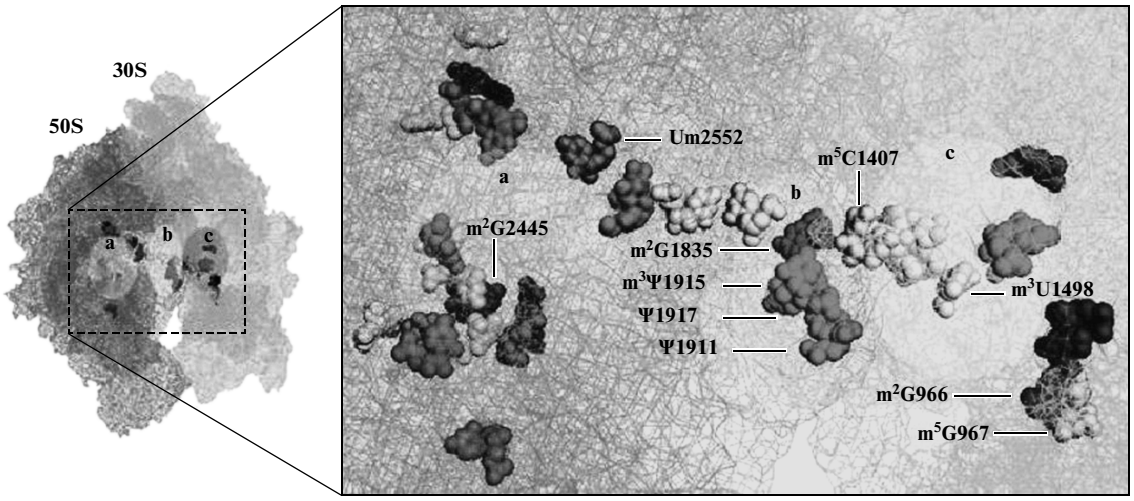

Fig. 13. Distribution of modified nucleotides in the 70S ribosome. Functionally important parts of the ribosome: the 50S subunit is dark gray; the 30S subunit is gray; a, peptidyltransferase center; b, contact of the two subunits; c, decoding center. Several of the most important modified nucleotides are indicated with arrows (modified from [45]).

Modifying enzymes utilize rRNAs, assembly intermediates, and complete ribosomal subunits as substrates. The majority of nucleotides subject to modification are conserved, but their functions are still incompletely understood. Modification changes the chemical properties of nucleotides, thus potentially affecting ribosome assembly. For instance, the hydrophobic group introduced by methylation may affect the stability of rRNA folding. Pseudouridylation, which increases hydrophilicity and improves stacking, acts possibly as a molecular glue to stabilize the functional RNA conformation, which is important for subsequent ligand orientation during translation. As a possible function, rRNA modification may provide

checkpoints for subunit assembly because each of the modifying enzymes prefers a relatively narrow range of assembly intermediates [46].

Like rRNAs, some of the ribosomal proteins were found to undergo enzymatic modification [47–53]. S11, L3, L7/L12, L16, and L33 contain monomethylated amino acid residues. L11 has three trimethylated residues. S5, S18, and L12 include acetylated residues. The acetylated form of L12 is historically termed L7. The L7/L12 proportion depends on the cell growth phase [54]. Small-subunit S6 is modified with four additional glutamic acid residues, and S11 is not only methylated, but half of S11 molecules contain isoaspartate as well [53].

Modifications of ribosomal components may act as molecular switches in some cases. For instance, KsgA methylatransferase modifies A1518 and A1519 in the 16S RNA only when assembly is completed successfully, providing a checkpoint for small-subunit assembly [56].

CONCLUSIONS

More than 50 years elapsed since the ribosome had been discovered and its structure and function had come to be investigated. In vitro reconstruction was initially used to study the ribosome composition and assembly. Such studies yielded Nomura's maps and revealed the order of ribosomal protein binding. Better knowledge of ribosome assembly was gained as structural methods developed [1]. Yet researchers still find new factors involved in ribosome assembly in stress, and the roles of assembly intermediates is still incompletely understood. Designing antibacterial drugs that suppress ribosome assembly in bacteria is an interesting problem.

REFERENCES

1. Yusupov M. 2001. Crystal structure of the ribosome at 5.5 angstrom resolution. *Science*. **292**, 883–896.
2. Wimberly B. 2000. Structure of the 30S ribosomal subunit. *Nature*. **407**, 327–339.
3. Cerretti D. 1983. The *spc* ribosomal protein operon of *Escherichia coli*: Sequence and cotranscription of the ribosomal protein genes and a protein export gene. *Nucleic Acids Res*. **11**, 2599–2616.
4. Paul B. 2004. rRNA transcription in *Escherichia coli*. *Annu. Rev. Genet*. **38**, 749–770.
5. Cannone J. 2002. The comparative RNA web (CRW) site: An online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. *BMC Bioinform*. **3**, 2.
6. Wimberly B. 2000. Structure of the 30S ribosomal subunit. *Nature*. **407** (6802), 327–339.
7. Sykes M. 2009. Complex assembly landscape for the 30S ribosomal subunit. *Annu. Rev. Biophys*. **38**, 197–215.
8. Williamson J. 2003. After the ribosome structure: How are the subunits assembled? *RNA*. **9**, 165–167.
9. Liiv A. 2004. Importance of transient structures during post-transcriptional refolding of the pre-23S rRNA and ribosomal large subunit assembly. *J. Mol. Biol*. **342**, 725–741.
10. Young R. 1978. Complementary sequences 1700 nucleotides apart from a ribonuclease III cleavage site in *Escherichia coli* ribosomal precursor RNA. *Proc. Natl. Acad. Sci. U. S. A*. **75**, 3593–3597.
11. Ghora B. 1979. Identification of a novel RNA molecule in a new RNA processing mutant of *Escherichia coli* which contains 5S ribosomal RNA sequences. *J. Biol. Chem*. **254**, 1951–1956.
12. Li Z. 1995. The tRNA processing enzyme RNase T is essential for maturation of 5S RNA. *Proc. Natl. Acad. Sci. U. S. A*. **92**, 6883–6886.
13. Shajani Z. 2011. Assembly of bacterial ribosomes. *Annu. Rev. Biochem*. **80**, 501–526.
14. Held W. 1973. Reconstitution of *Escherichia coli* 30S ribosomal subunits from purified molecular components. *J. Biol. Chem*. **248**, 5720–5730.
15. Jagannathan I. 2003. Assembly of the central domain of the 30S ribosomal subunit: roles for the primary binding ribosomal proteins S15 and S8. *J. Mol. Biol*. **330**, 373–383.
16. Jagannathan I. 2004. Ribosomal protein dependent orientation of the 16S rRNA environment of S15. *J. Mol. Biol*. **335**, 1173–1185.
17. Nierhaus K. 1980. The assembly of the prokaryotic ribosome. *BioSystems*. **12**, 273–282.
18. Mulder A. 2010. Visualizing ribosome biogenesis: parallel assembly pathways for 30S subunit. *Science*. **330** (6004), 673–677.
19. Chen S. 2012. Characterization of the ribosome biogenesis landscape in *E. coli* using quantitative mass spectrometry. *J. Mol. Biol*. **425** (4), 767–779.
20. Held W. 1973. Rate determining step in the reconstitution of *E. coli* 30S ribosomal subunits. *Biochemistry*. **12**, 3273–3281.
21. Bunner A. 2010. The effect of ribosome assembly cofactors on in vitro 30S subunit reconstitution. *J. Mol. Biol*. **398**, 1–7.
22. Talkington M. 2005. An assembly landscape for the 30S ribosomal subunit. *Nature*. **438**, 628–632.
23. Nierhaus K. 1974. Total reconstitution of functionally active 50S ribosomal-subunits from *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A*. **71**, 4713–4717.
24. Sieber G. 1978. Kinetic and thermodynamic parameters of assembly in vitro of large subunit from *Escherichia coli* ribosomes. *Biochemistry*. **17**, 3505–3511.
25. Hayes F. 1971. Biosynthesis of ribosomes in *E. coli*: 1. Properties of ribosomal precursor particles and their RNA components. *Biochimie*. **53**, 369–382.
26. Lindahl L. 1973. Two new ribosomal precursor particles in *E. coli*. *Nature New Biol*. **243**, 170–172.
27. Lindahl L. 1975. Intermediates and time kinetics of in vivo assembly of *Escherichia coli* ribosomes. *J. Mol. Biol*. **92**, 15–37.
28. Tanner N. 2003. The Q motif: A newly identified motif in DEAD box helicases may regulate ATP binding and hydrolysis. *Mol. Cell*. **11**, 127–138.

29. Elles L. 2009. A dominant negative mutant of the *E. coli* RNA helicase DbpA blocks assembly of the 50S ribosomal subunit. *Nucleic Acids Res*. **37**, 6503–6514.
30. Charollais J. 2003. The DEAD-box RNA helicase SrmB is involved in the assembly of 50S ribosomal subunits in *Escherichia coli*. *Mol. Microbiol*. **48**, 1253–1265.
31. Charollais J. 2004. CsdA, a cold-shock RNA helicase from *Escherichia coli*, is involved in the biogenesis of 50S ribosomal subunit. *Nucleic Acids Res*. **32**, 2751–2759.
32. Jagessar K. 2010. Functional and molecular analysis of *Escherichia coli* strains lacking multiple DEAD-box helicases. *RNA*. **16**, 1386–1392.
33. Jain C. 2008. The *E. coli* RhIE RNA helicase regulates the function of related RNA helicases during ribosome assembly. *RNA*. **14**, 381–389.
34. Al Refaii A. 2009. Ribosome biogenesis is temperature-dependent and delayed in *Escherichia coli* lacking the chaperones DnaK or DnaJ. *Mol. Microbiol*. **71**, 748–762.
35. El Hage A. 2001. The chaperonin GroEL and other heat-shock proteins, besides DnaK, participate in ribosome biogenesis in *Escherichia coli*. *Mol. Gen. Genet*. **264**, 796–808.
36. Alix J. 1993. Mutant DnaK chaperones cause ribosome assembly defects in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A*. **90**, 9725–9729.
37. Olins P.O. 1981. Translational regulation by ribosomal protein S8 in *Escherichia coli*: Structural homology between rRNA binding site and feedback target on mRNA. *Nucleic Acids Res*. **9**, 1757–1764.
38. Britton R. 2009. Role of GTPases in bacterial ribosome assembly. *Annu. Rev. Microbiol*. **63**, 155–176.
39. Himeno H. 2004. A novel GTPase activated by the small subunit of ribosome. *Nucleic Acids Res*. **32**, 5303–5309.
40. Hwang J. 2010. A bacterial GAP-like protein, YihI, regulating the GTPase of Der, an essential GTP-binding protein in *Escherichia coli*. *J. Mol. Biol*. **399**, 759–772.
41. Hwang J. 2001. An essential GTPase, Der, containing double GTP-binding domains from *Escherichia coli* and *Thermotoga maritima*. *J. Biol. Chem*. **276**, 31415–31421.
42. Hwang J. 2006. The tandem GTPase, Der, is essential for the biogenesis of 50S ribosomal subunits in *Escherichia coli*. *Mol. Microbiol*. **61**, 1660–1672.
43. Jiang M. 2006. The *Escherichia coli* GTPase CgtAE is involved in late steps of large ribosome assembly. *J. Bacteriol*. **188**, 6757–6770.
44. Woodson S. 2008. RNA folding and ribosome assembly. *Curr. Opin. Chem. Biol*. **12** (6), 667–673.
45. Chow C. 2007. Expanding the nucleotide repertoire of the ribosome with posttranscriptional modifications. *ACS Chem. Biol*. **2** (9), 610–619.
46. Hou Y. 2010. Stereochemical mechanisms of tRNA methyltransferases. *FEBS Lett*. **584**, 278–286.
47. Colson C. 1979. Genetics of ribosomal protein methylation in *Escherichia coli*: 3. Map position of two genes, prmA and prmB, governing methylation of proteins L11 and L3. *Mol. Gen. Genet*. **169**, 245–250.
48. Cashel M. 1996. Regulation of ribosome synthesis. In: *Escherichia coli and Salmonella: Cellular and Molecular Bology*, 2nd ed. Ed. Neidhardt F.C. Washington, DC: ASM Press.
49. David C. 1999. Isoaspartate in ribosomal protein S11 of *Escherichia coli*. *J. Bacteriol*. **181**, 2871–2877.
50. Arnold R. 1999. Observation of *Escherichia coli* ribosomal proteins and their posttranslational modifications by mass spectrometry. *Anal. Biochem*. **269**, 105–112.
51. Kowalak J. 1996. Methylthio-aspartic acid: identification of a novel posttranslational modification in ribosomal protein S12 from *Escherichia coli*. *Protein Sci*. **5**, 1625–1632.
52. Brosius J. 1976. The primary structure of protein L16 located at the peptidyltransferase center of *Escherichia coli* ribosomes. *FEBS Lett*. **68**, 105–109.
53. Holmes K. 2004. Mapping structural differences between 30S ribosomal subunit assembly intermediates. *Nature Struct. Mol. Biol*. **11**, 179–186.
54. Nesterchuk M. 2011. Posttranslational modifications of ribosomal proteins in *Escherichia coli*. *Acta Naturae*. **3** (2), 22–33.
55. Isono S. 1981. Ribosomal protein modification in *Escherichia coli*: 3. Studies of mutants lacking an acetylase activity specific for protein L12. *Mol. Gen. Genet*. **183** (3), 473–477.
56. Connolly K. 2008. Mechanistic insight into the ribosome biogenesis functions of the ancient protein KsgA. *Mol. Microbiol*. **70** (5), 1062–1075.

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